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Acquisition of Topical Resistance to Thermal Injury.* (20784)

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Decrease in non-specific inflammatory reaction of tissues repeatedly stimulated by non-living irritating material, has been demonstrated(1-5). In a previous paper(4), we reported that an inflammation, produced by the injection of various irritants into the paw of a rat, protected this paw against a subsequent inflammation. When, after the first local inflammation had subsided, a second generalized hyperergic reaction was produced by an intraperitoneal injection of dextran or egg-white, the characteristic inflammatory edema appeared in the snout, ears and paws, but not in the pre-irritated paw. This permitted us to exclude the direct involvement of higher systemic regulations and to demonstrate this type of protection as being strictly topical.

In the investigation reported here, an attempt was made to obtain some data on the actual state of non-specific "sensitivity" or "reactivity" of pre-irritated tissues to a new inflammatory stimulus. Heat was used for the second irritation and was always given under compression of the blood vessels, thus making the tissues "poikilothermal" and ensuring an absolute uniform irritation in normal and pre-irritated tissues. This procedure eliminated the possibility, existing after a repeated administration of a chemical irritant, that through changes in tissue structures, the irritant might be mechanically prevented from acting uniformly upon the tissues. In this study, the influence of a preceding inflammation on the reaction to "poikilothermal heating" was investigated. The preceding inflammation was induced by the injection of edema-producing irritants, such as dextran or egg-white, into the rat paw, or by rubbing croton oil into the skin of the rabbit ear. Further, the influence of a pre-irritation by poikilothermal heating on the effect of a second, more intense heating was studied. Finally, the problem was investigated as to whether a pre-irritation by heat gives protection also against the reaction to a subsequent irritation by edema-producing chemical irritants.

Methods. Animals: Male Sprague-Dawley rats, weighing between 100 and 120 g, and male albino rabbits of 1.5 to 2.0 kg, were used. *Irritants.* Dextran was given in a saline dilution 1:37.5 of the commercial 6% solution (Macrodex). For local irritation, 0.2 ml was injected beneath the plantar aponeurosis of the hind paw of a rat; generalized hyperergic reaction was produced by the intraperitoneal injection of 1.0 ml. Egg-white was given in a saline dilution 1:5, 0.2 ml for local irritation, 1.0 ml intraperitoneally for generalized reaction. Croton oil, diluted 1:5 with oil (Mazola), was rubbed into the skin of the rabbit ear (2 drops on the upper and 2 drops on the lower surface) using rubber gloves. *"Poikilothermal heating." In rats.* Under compression of the aorta abdominalis with a rubber tube, the right hind paw was placed into 48°C water for 2 minutes, 15 seconds; the compression was then immediately released. For the subsequent irritation (the

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TABLE I. Topical Protection against Hyperergic Reaction to Dextran or Egg-White by a Preceding Inflammation in the Rat Paw; No Protection against Poikilothermal Heating. (6 rats in each group.)

	Paw	1st irritation (subcut.)	2nd irritation	Inflammatory index	% degree of protection in right paw
I	R*	.2 ml dextran	Heating both paws	48.5 ± 10.4	No protection
	L*	—	(48°C, 1'30") †	37.8 ± 5.9	
II	R	*idem*	(*idem* for 2'15") †	148.0 ± 18.7	" "
	L	—		143.0 ± 16.8	
III	R	*idem*	Egg-white, 1 ml intraper. †	5.5 ± 5.0	97.8 ± 2
	L	—		235.2 ± 28.7	
IV	R	.2 ml egg-white	Heating both paws (49°C,	306.5 ± 20.6	1.0 ± 1.7
	L	—	2 min.) 24 hr later	310.5 ± 23.0	
V	R	*idem*	*idem* 48 hr later	312.8 ± 17.2	4.8 ± 3
	L	—		336.3 ± 16.8	
VI	R	*idem*	Dextran, 1 ml intraper.	5.8 ± 2.5	95.2 ± 3
	L	—	33 hr later	137.0 ± 31.2	

* R = Right; L = Left.
† 36 hr later.

time interval between the two irritations is individually indicated in the tables and legends), both hind paws, again under compression of the aorta, were placed into 49°C water for 2 minutes and the compression released immediately after. In order to minimize the differences in the duration of maximal tissue temperature which might occur if the volumes of the hind paws differed, the subsequent heating was given for 4 minutes at 47°C, if the right hind paw was still showing an increase in diameter due to the previous exposure to heat. By measuring the increase in the dorso-plantar diameter of the hind paws, at the level of the metatarsus, several times, before, during, and after the maximum reaction, an arbitrary inflammatory index (Σ% increase in diameter of all measurements) was determined for each paw. The comparison of the indices in the hind paws of the same animal permitted the assessment of the degree of protection as a percentage.

In rabbits. The volume of both ears was measured, after shaving, by the water displacement in a test tube. The right ear was then placed into 49°C water for 2 minutes under compression of the blood vessels at the base of the ear, with rubber-armed curved arterial clamps. Immediately afterwards, the compression was released. (As there are differences in the reactions to this treatment in rabbits of varying ages and races, and at different room temperatures, the duration of the first heating should be arranged so that the inflammation does not exceed a 50% increase in ear volume, and so that the volume is almost back to normal after 2 days.) For the subsequent heating, both ears were clamped and placed, simultaneously into 49°C water for 2 minutes, by holding the animal horizontally upside down. The compression was released immediately afterwards. Estimations of the reaction and the degree of protection were made on the basis of the volume measurements in analogy with the procedure used for the rat.

Results. Topical protection against inflammation. A local inflammatory edema was elicited by the injection of dextran or egg-white into the right hind paw of rats (Table I). After gross signs of inflammation had disappeared, a second generalized hyperergic reaction in all 4 paws was produced by intraperitoneal injection of egg-white or dextran (Groups III and VI). In the right paw, previously subjected to an egg-white or dextran inflammation, the inflammatory edema did not occur. The preceding inflammation, however, gave no protection against a subsequent inflammatory reaction to heating, even if the heat irritation was very mild (Groups I and II), or made at different time intervals after the first inflammation (Groups IV and V). Also, an inflammation produced in the rabbit

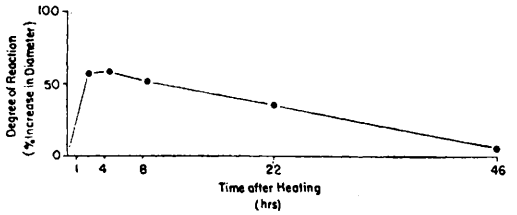

FIG. 1. Inflammatory reaction in hind paw of rats submitted to a poikilothermal heating, such as used for the pre-irritation in the following series of experiments (48°C, 2 min. 15 sec.). (Group of 6 rats.)

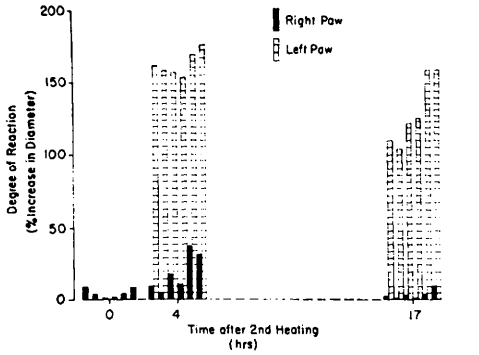

FIG. 2. Topical protection against heat injury by pre-irritation with heat. Inflammatory reaction in both hind paws of rats, submitted to poikilothermal heating (49°C, 2 min.). Right hind paw pre-heated (48°C, 2 min. 15 sec.), 2 days in advance. (Group of 6 rats.)

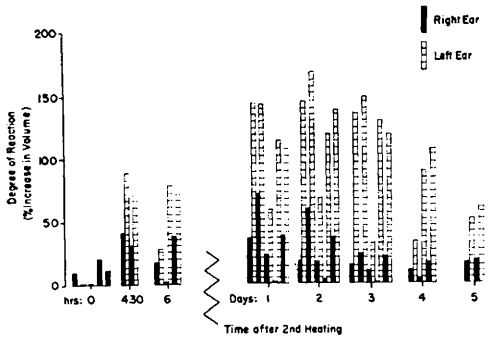

FIG. 3. Topical protection against inflammatory reaction to poikilothermal heating (49°C, 4 min.), in the rabbit ear. Right ear pre-heated (49°C, 2 min.), 3 to 5 days in advance. (Group of 5 rabbits.)

ear (5 rabbits) by rubbing it with croton oil, thus leading to a considerable swelling and to some superficial epithelial lesions, did not reduce the degree of a subsequent inflammatory reaction to heating (49°C, 4 minutes, 3-12 days later), given after healing of the epithelial defects and after disappearance of macroscopical signs of inflammation. The inflammatory index in the right pre-irritated ear was 634.0 ± 36.0, and 543.2 ± 50.0 in the left, non-pretreated ear. However, when tissues were pre-irritated by a mild heating, a marked topical resistance against a new and more severe heat injury was observed. The average degree of the inflammatory reaction following such a pre-irritation of a rat paw, is shown in Fig. 1. Sometimes, after pre-irritation, tissues showed only minimal signs of inflammation, even though a topical resistance against a new heat injury developed.

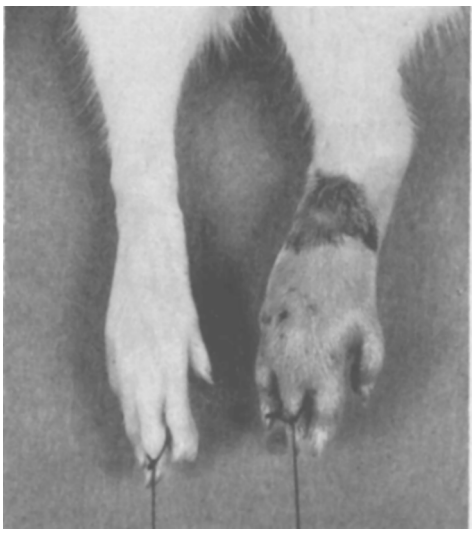

FIG. 4. Topical protection against necrosis. Poikilothermal heating of both hind paws (49°C, 2 min. 30 sec.). Right hind paw pre-heated (48°C, 2 min. 15 sec.), 3 days in advance.

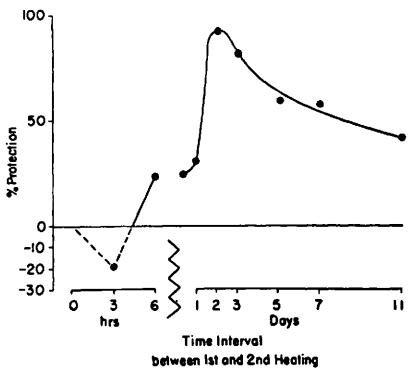

FIG. 5. Duration of the topical protection against inflammatory reaction to heat (47°C, 4 min. in the groups heated 3 hr, 6 hr and 1 day after pre-irritation, 49°C, 2 min. for the rest). Pre-irritation: 48°C, 2 min. 15 sec. (Groups of 6 rats each.)

TABLE II. Topical Protection against Hyperergic Reaction to Dextran and Egg-White by Poikilothermal Pre-Irritation. (Groups of 6 rats each.)

	Paw	1st irritation	2nd irritation (3 days later)	Inflammatory index	% degree of protection in right paw
I	R*	48°C, 2'15"	Dextran, 1 ml intraper.	5.0 ± 2.1	96.6 ± 1.4
	L*	—		132.8 ± 27.0	
II	R	*idem*	Egg-white, 1 ml intraper.	12.0 ± 8.9	93.3 ± 2.6
	L	—		157.5 ± 31.6	
III	R	*idem*	Dextran, .2 ml subcut.	265.0 ± 19.5	21.3 ± 3.2
	L	—	*idem*	346.0 ± 18.2	

* R = Right; L = Left.

The degree of this protection in the rat paw is shown in Fig. 2. Under these experimental conditions, the reaction in both paws was at the maximum approximately 4 hours after the second heating. Further, if a rabbit ear is pre-irritated by poikilothermal heating, it becomes, after macroscopical normalization, much less reactive to a new heat exposure (Fig. 3).

Protection against necrosis. If, by extending the duration of heating, the degree of tissue irritation is increased to a point where necrosis develops in the normal rat paw, the pre-irritated right paw will show but a mild inflammatory reaction and no necrosis (Fig. 4). Protection against the development of necrosis was observed also in the rabbit ear. Here, too, the right ear, pre-irritated by heating, showed only a mild reaction, whereas the normal left ear became necrotic if, in the second step, the duration of heating was extended or when an increased room temperature sometimes led to a stronger reaction to the second heating.

Duration of protection. The duration of the topical resistance after thermal pre-irritation against a new heat injury in the rat paw, is shown in Fig. 5. The protection appears to develop rapidly. After reaching a maximum, 2 days after the first irritation, the protection decreases in the form of a logarithmic curve.

Non-specificity of protection. The inflammatory edema formation, after intraperitoneal injection of dextran or egg-white in rats (Table II), is clearly inhibited in the paw which has been previously given a poikilothermal heating (Groups I and II). This may be due chiefly to a mechanical barrier of new structural elements which remained in the tissues after the first inflammation, and which, however, were almost ineffective against the concentrated action of locally injected dextran (Group III). Dextran, injected locally, produced an almost equal degree of inflammation in normal and in pre-heated paws. The ability of a pre-heated paw to react with acute edema formation can therefore be considered as being practically unchanged.

Discussion. There seems to be little doubt that heating under compression of the blood vessels brings both normal and pre-irritated tissues to the same temperature, over the same length of time. However, the tissues pre-irritated by heat show considerably less or almost no reaction, in comparison to normal non pre-irritated tissues, if an equal poikilothermal heating is given to both at the same time. Hence, it can be assumed that a mild pre-irritation by heat has produced some basic functional changes in the tissues thus making them indifferent towards the action of heat, which leads to inflammation and necrosis in normal tissues. Neither pre-irritation of tissues by the injection of irritants such as dextran or egg-white, leading to an acute edematous inflammation, nor a pre-treatment with croton oil, leading to a more chronic inflammation, provide protection against a subsequent poikilothermal heating and apparently do not produce such functional changes as ensue after pre-irritation by heat. This topically acquired indifference of tissues towards the action of heat, is probably a biological phenomenon similar to that of the adaptation to higher temperatures of lower forms of life, *e.g.*, Paramoecium and flagellates (6-8), by gradual exposure.

The tissue histamine content, even though decreased after poikilothermal pre-irritation in the skin of the rat paw, does not seem to be directly responsible for the protection against a new heating, since a depletion of the histamine in the rat paw by locally injecting compound 48/80 according to Feldberg and Talesnik(9), did not result in any protection against poikilothermal heating(10).

Protection against fatal shock in mice due to tourniquet of the hind legs has been reported when the same legs were submitted to a non-fatal tourniquet, 21 to 38 days in advance(11). In dogs and calves, subjected to burns, a rise in aminopeptidase activity was found in the lymph draining the burned area (12). Further, during the experiments reported here, it was found that the formation of hemolymphnodes in the popliteal region, generally occurring half an hour after poikilothermal heating of the rat paw and thus preceding the inflammatory swelling, was less or did not occur at all after the heating of the protected, pre-heated paw.‡ India ink particles, however, after poikilothermal heating, moved faster in the lymph vessels of the protected leg than they did in the normal side(13).

Therefore, it may be concluded that an irritation by heat leads to the exhaustion or blocking of a mechanism by which cytolytic substances are liberated from the injured tissues, or leads to the blocking of such substances. Thus, the reported data seem to give new emphasis to the findings of Beloff and Peters(14), who observed that a proteolytic enzyme, found in the skin of rats, was decreased after heating, presumably, according to the authors, because of an increased escape of the proteinase from the cells.

After local injection of egg-white or dextran into the rat paw, the inflammatory edema reaches its maximum within 20-30 minutes, while, after heating, this takes 3-4 hours, and thus the development of the inflammatory edema is here apparently dependent upon intermediary substances liberated in the tissues. Hence, the relatively unimpaired ability of pre-heated tissues to react with an acute edema formation as in the case of locally injected dextran, adds by exclusion further support to the assumption that the mechanism of protection against heat injury is mainly on the level of substances being dislocated and/or liberated normally after heat injury and inducing inflammation and necrosis.

‡ Hemolymphnodes were never observed after injection of egg-white or dextran into the rat paw.

Summary. 1. A topically acquired indifference of tissue cells towards the action of heat, such as leads to inflammation and necrosis in normal tissues, is described. This indifference was produced by a mild local pre-irritation of tissues with heat (immersion of a rat paw in 48°C water for 2 minutes, 15 seconds, or of a rabbit ear in 49°C water for 2 minutes). Heating was always done under compression of the blood vessels. 2. Local pre-irritation obtained by the injection of dextran or egg-white into the rat paw, or by producing a more chronic inflammation with croton oil applied on the rabbit ear, was ineffective in protecting the tissues locally against a subsequent heat exposure given under compression of the blood vessels. 3. After local pre-irritation of the rat paw by heat, a topical protection was observed also against the inflammatory reaction to intraperitoneally injected dextran or egg-white and, to a slight degree, to dextran subcutaneously injected into the paw.

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Blood Levels and Urinary Excretion in Normal Subjects after Ingestion of Tetracycline Analogues.*† (20785)

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The antibacterial action of the 3 chemically closely related antibiotics—tetracycline, oxytetracycline (Terramycin), and chlortetracycline (Aureomycin)—was found to be very similar against most of the strains of common human pathogenic bacteria that were tested (1). The results of assays of blood and urine for antibiotic activity following oral administration of these 3 agents are presented in this paper.

Materials and methods. Tetracycline (Achromycin) and chlortetracycline (Aureomycin) were supplied by Dr. Stanton M. Hardy of the Lederle Laboratories, and oxytetracycline (Terramycin) was provided by Dr. Gladys L. Hobby of Chas. Pfizer & Co. Each of these antibiotics was furnished in both capsules and tablets containing 250 mg equivalent of the free base; all were in the form of the hydrochlorides, except the oxytetracycline tablets, which were of the free base. The subjects were male members of the hospital staff who had not received any antibiotics for several weeks prior to the beginning of the study. Assays for antibiotic activity were done on citrated plasma from venous blood and on "clean" voided specimens of urine; 2-fold dilutions were made in brain-heart infusion broth (Difco pH ± 7.4) and seeded with an equal volume of a 10^{-4} dilution of an overnight growth of *B. cereus* No. 5 (obtained from Dr. A. C. Dornbush of Lederle Laboratories). The tests with plasma were read for visible growth after incubation at 37°C for 18 hours (and in some instances they were examined again after 24 and 40 hours); assays of urine were observed at 8, 18, and 24 hours. The results were recorded as the maximum final dilution completely inhibiting growth, as indicated by absence of visible clouding of the medium. The minimum inhibiting concentration in the specimens was determined by comparison with freshly prepared standard solutions assayed simultaneously and read at 18 hours.

Results. Two separate experiments were carried out. In the first, 12 subjects were given 1.0 g of each of the 3 analogs in rotation 4 to 7 days apart, 2 receiving capsules and 2, tablets of one of the 3 tetracyclines on each of the 3 days. None of the participants knew which of the analogs were given until the entire experiment was completed. Plasma and urine were collected at specified intervals, and those obtained at each interval were assayed simultaneously so that samples from all the subjects who had taken each of the 3 antibiotics were included in each test. The levels of antibiotic in the plasmas are shown in Fig. 1. None of those obtained before the doses showed inhibition of the test organism. More than one-half of the subjects had no demonstrable level at one hour, but the levels increased to a maximum between 2 and 6 hours. Two subjects who received tablets of oxytetracycline base showed no antibiotic

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† Summary of some of these findings presented at the Antibiotic Symposium, Washington, D. C., Oct. 28, 1953.